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CTI-N5 DIV-12

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| Applicant | : | Weiss *et al.* |
| Serial No. | : | 08/486,313 |
| Filed | : | June 7, 1995 |
| For | : | MULTIPOTENT NEURAL STEM CELL COMPOSITIONS |
| Examiner | : | A-M. Baker |
| Group Art Unit | : | 1632 |

Honorable Assistant Commissioner for Patents
Washington, D.C. 20231

Providence, Rhode Island

**DECLARATION UNDER 37 C.F.R. § 1.132**

I, JOSEPH P. HAMMANG, hereby declare and state as follows:

1. I received my B.S. and M.S. (Zoology) degrees from the University of Wisconsin, Oshkosh, Wisconsin in 1980 and 1982, respectively. I received my Ph.D. (Neuroscience) from the University of Wisconsin, Madison, Wisconsin in 1990. I am a named inventor on this application. I have been working in the field of cell and molecular neurobiology and with myelin mutant animals since 1982, and working with neural stem cells since 1991.

2. I understand that the pending claims are directed to methods for transplanting neural stem progeny into a host.

3. I am aware of the Examiner's May 4, 2000 Advisory Action, and in particular I understand that the Examiner has rejected the pending claims under 35 U.S.C. §112 contending that "[t]he claims are not enabled because the transplantation of multipotent neural stem cell progeny into a host has not been demonstrated to provide any therapeutic benefit to the host."

4. I make this declaration to rebut the Examiner's rejection, which I do not agree with. In view of the express statements in the specification regarding transplantation of neural stem cell cultures and the voluminous experimental evidence that has been accumulated, in my opinion,

the ordinarily skilled artisan would be able to routinely transplant the described neural stem cell cultures with a reasonable expectation of success.

5. The neural stem cells described for the first time in this invention, and the ability for the art (provided by the inventors here) to obtain proliferating cultures of those cells, has been widely hailed as a landmark in neurobiology. Prior to this invention, the operating dogma in neurobiology was that the brain was relatively quiescent, and that there was no "stem cell" that could be proliferated and then differentiated to form the three major cell types in the central nervous system (i.e., neurons, astrocytes and oligodendrocytes). In related applications, the inventors have claimed such neural stem cell cultures, methods of proliferating them, methods for inducing such cultures to form dopaminergic neurons, as well as methods for genetically modifying them. Those patents have been issued by the USPTO (see, e.g., US Patent Nos. 5,750,376; 5,981,165; 5,980,885; 5,851,832; and 6,071,889). In the instant related application (which is in the same chain of priority and has the same specification as the foregoing issued patents), we are claiming methods for transplanting those neural stem cell cultures into hosts.

6. First, we expressly stated in the specification that the neural stem cell cultures of this invention are particularly suited for transplantation, since, until now, cultures of proliferating neural cells have not been available to the art. Further, with our invention, the tissue source is well-defined, reproducible, and is not derived from an oncogene-immortalized cell line (thus being non-tumorigenic). *See,* specification, pg. 11, lines 15-20. In fact, another of the named inventors, Dr. Baetge, has concluded that such neural stem cell cultures are) "ideal" for nervous system transplantation. See, *Baetge et al.,* 695 Ann N.Y. Acad. Sci., pp. 285-291 (1993).

7. Second, we also expressly provided ample guidance in the specification about how to transplant CNS neural stem cells (*see,* specification, pg. 36, line 10, to pg. 42, line 13; pg. 68, line 16, to pg. 69, line 18; pg. 78, line 17, to pg. 71, line 6; pg. 96, line 12, to pg. 97, line 28). And, we even provided working examples of neural stem cell transplantation in various disease

models, including Huntington's disease, Parkinson's disease, and cardiac arrest. *See,* e.g., specification, pp. 96-101.

8. Third, and of particular relevance the Examiner's comments, following transplantation, our neural stem cell cultures have been shown to actively secrete cellular products, such as peptides and have been shown to differentiate *in vivo* into tissue-environment appropriate cell lineages that may replace diseased or damaged tissue or may repair such tissues. This is both detailed in the specification (*see,* specification, pg. 69, line 19, to pg. 70, line 7; pg. 97, line 29, to pg. 103, line 14) and in subsequent scientific publications (by us and other groups). I have detailed this evidence below. Most importantly, however, these two facts -- (1) that the transplanted cells secrete cellular products, and (2) that the neural stem cell cultures exhibit tissue-specific differentiation upon transplantation, in my view, would inescapably lead the ordinarily skilled artisan to conclude that transplantation of such neural stem cell cultures would have a reasonable expectation of success in providing a therapeutic benefit to the host. There is not, in my view, any objective evidence that could lead the ordinarily skilled artisan to conclude otherwise.

9. In my own work, I have demonstrated that transplanted CNS neural stem cell cultures express *lacZ* and appropriately form myelinating oligodendrocytes *in vivo* in dys-myelinated environments (such as the well accepted myelin-deficient rat model). *See,* Hammang *et al.*, in 21 *Methods in Neurosciences*, 281-293 (Flanagan *et al.*, eds., Academic Press, San Diego, 1994) ("*Hammang I*"); Hammang *et al.*, 147(1) Exp Neurol. 84-95 (1997) (*Hammang II*). The results obtained in these studies confirm, to me, that because the cultures can continue to produce cellular substances when transplanted and those cell make appropriate lineage decisions *in vivo*, transplantation of such neural stem cell cultures would be useful in providing a therapeutic benefit to the host, as originally stated in the specification.

10. Other subsequent work also supports this conclusion. For example, Milward *et al.*, 50 J. Neurosci. Res. 862-871 (1997) ("*Milward*") successfully transplanted canine CNS neural stem

cells both into rat and into a shaking (sh) pup myelin mutant dog (a model of human myelin diseases). In *Milward*, canine neural stem cell cultures were transplanted into the myelin-deficient (*md*) rat spinal cord, resulting in the production of myelin by graft-derived cells (*see, Milward,* pg. 868, col. 1, 2nd para.), demonstrating that transplanted CNS neural stem cells can differentiate in the recipient to form myelin-producing oligodendrocytes and therapeutically provide myelin to recipients.

Zhang *et al.*, 96 Proc. Natl. Acad. Sci. USA 4089-94 (1999) ("*Zhang*") reports similar results, in which neural stem cell cultures were generated from both juvenile and adult rats and used to produce myelin-forming cells, and when transplanted into *md* rats, those cells produced "robust myelination". *See, Zhang*, pp. 4093-94. This too demonstrates that transplantation of such neural stem cell cultures would be useful in providing a therapeutic benefit to the host.

Likewise, Brüstle *et al.*, 16 Nature Biotechnol., pp. 1040-1044 (1998) ("*Brüstle*") describes the implantation of fetal human CNS progenitor cells into mice that "acquire an oligodendroglial phenotype and participate in the myelination of host axons". All of these publications demonstrate that transplantation of such neural stem cell cultures would be useful in providing a therapeutic benefit to the host, as originally stated in the specification.

11. The *Milward* results are particularly demonstrative. The shaking pup canine model is a particularly harsh model of dys-myelination. The pups are born with little to no myelin sheath. The *Milward* data demonstrate that that the present invention can be used to provide myelination in an almost myelin-free *in vivo* environment. In addition, as *Milward* reports, the grafted cells had integrated normally into the adult *sh* pup cytoarchitecture. See, Ex. 2, p. 867, right column. This is powerful support that transplantation of such neural stem cell cultures would be useful in providing a therapeutic benefit to the host. This is particularly true in "real-life" disease situations where de-myelination is a relatively slow process occurring not globally but in patches.

12. Yandava *et al.*, 96(12) Proc. Natl. Acad. Sci., pp. 7029-34 (1999) ("*Yandava*") also presents data that demonstrates a therapeutic effect upon transplantation. *Yandava* showed that

transplantation of CNS neural stem cells results in "global" cell replacement and therapeutically effective remyelination in mice. *Yandava* showed that neural stem cells transplanted at birth resulted in widespread engraftment throughout the dysmyelinated shiverer (*shi*) mouse brain with repletion of myelin basic protein (MBP). *Yandava* showed that a number of recipient animals evinced decrement in their symptomatic tremor -- a clear therapeutic benefit.

13. Others have also demonstrated that our neural stem cell cultures when transplanted are in fact capable of replacing the critical functions of lost or deficient neural populations. See, e.g., Flax *et al.*, 16 Nature Biotechnol., pp. 1033-1039 (1998) ("*Flax*"). *Flax* showed that transplantation of CNS neural stem cells provides a therapeutic benefit in the meander tail (*mea*) mouse, a mouse mutant model that is characterized by a cell-autonomous failure of granule neurons to develop or survive in the cerebellum, especially the anterior lobe. *Flax* transplanted human CNS neural stem cells into newborn *mea* cerebella and confirmed that the human neural stem cells provided "replacement neurons" with the "definitive size, morphology, and location of cerebellar granule neurons (Fig. 6E-G)" (*Flax*, pg. 1037, col. 2,2$^{nd}$ para). Thus, *Flax* showed that transplanted CNS neural stem cells can differentiate in the recipient to form granule neurons and therapeutically provide replacement neurons to recipients (such as these *mea* mice). This, in my view is unequivocal evidence of a therapeutic benefit upon transplantation of neural stem cell cultures into a host, as claimed here. My view is also supported independently by other workers in the field who have reviewed the Flax, Nature Biotech paper. See Zigova & Sanberg, 16 Nature Biotechnol., pp. 1007-1008 (1998) ("*Zigova & Sanberg*") which states that the *Flax* data "provides strong evidence that the NSCs [neural stem cells] are able to perform *in vivo* and *in vivo* all the critical functions previously described for their rodent counterparts" (*Zigova & Sanberg*, pg. 1007, middle column).

14. One important characteristic of the cells claimed in this invention, is their ability, unlike cells in methods in the prior art, to migrate to the desired target and differentiate into the appropriate lineage. As noted above, and additionally in Fricker *et al.*, 19 J. Neurosci., pp. 5990-6005 (1999) ("*Fricker*"), the claimed method are particularly ideal as therapeutics for precisely

this reason. *Fricker* showed that when CNS neural stem cells were transplanted into neurogenic regions in the adult rat brain, the subventricular zone, and hippocampus, the *in vitro* propagated cells migrated specifically along the routes normally taken by endogenous neuronal precursors: along the rostral migratory stream to the olfactory bulb and within the subgranular zone in the dentate gyrus, and exhibited site-specific neuronal differentiation in the granular and periglomerular layers of the bulb and in the dentate granular cell layer. The CNS neural stem cells exhibited substantial migration also within the non-neurogenic region, the striatum, and showed differentiation into both neuronal and glial phenotypes. In short *Fricker* showed the ability of the human neural stem cells to respond *in vivo* to guidance cues and signals that can direct their differentiation along multiple phenotypic pathways. For this reason, *Fricker* too, in my view, demonstrates that transplantation of such neural stem cell cultures would be useful in providing a therapeutic benefit to the host, as originally stated in the specification.

15. Finally, I would like to draw a recent publication to the Examiner's attention that I believe also is unequivocal -- demonstrating that transplanted neural stem cell cultures can both continue to express a foreign gene and migrate in a site specific fashion in host tissue. See, e.g., Aboody et al., 97 Proc. Natl. Acad. Sci. USA, pp. 12846-51 (November 2000) ("*Aboody*"). In view of these results, *Aboody* also expressly states (see, e.g., throughout the paper, and particularly Abstract and p. 12851) that neural stem cell cultures provide a transplantation "platform" since upon transplantation those cells can both continue to express a foreign gene and migrate in a site specific fashion in host tissue for "dissemination of therapeutic genes". Thus, *Aboody* too, in my view, demonstrates that transplantation of such neural stem cell cultures would be useful in providing a therapeutic benefit to the host, as originally stated in the specification.

16. For all the foregoing reasons, I believe that the Examiner should withdraw the rejection and allow the pending claims.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

Joseph P. Hammang

Signed at Providence, Rhode Island
this 16th day of December, 2000

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